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Research Article

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Synthesis of 7-Chloro-2-[3-(4-Chlorophenyl)-5-Aryl-4,5-Dihydro-1H-Pyrazol-1-Yl]-6-Fluro-1,3-Benzothiazoles and 7-Chloro-2-[3-(4-Chlorophenyl)-5-Aryl-1H-Pyrazol-1-Yl]-6-Fluoro-1,3-Benzothiazoles and their Antimicrobial, Anti-Inflammatory and Analgesic Activity

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ABSTRACT

In continuation of our search for pharmaceutically active heterocyclic compounds we undertook the synthesis of 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl]-6-fluro-1,3-benzothiazoles (5) and 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazoles (6). The required 2-hydrazinobenzothiazole (3) was prepared by the action of 2-amino-7-chloro-6-fluro benzothiazole and hydrazine hydrate. Different chalcones (4a-h) have been obtained by the condensation of substituted benzaldehydes with p-chloroacetophenone which were then treated with (3) to obtain corresponding 7-chloro-2-[3-(4-chlorophenyl)-5aryl-4,5-dihydro-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazoles (5a-h). The compounds 5 were then converted into 7-chloro-2-[5-aryl-3-(4-chlorophenyl)-1H-pyrazol-1-yl]-6-fluro-1,3-benzothia-zoles (6a-h) by treating with Iodobenzene diacetate in dichloromethane at room temperature. All the compounds synthesized were characterized by spectral data. All the new compounds were screened for anti-inflammatory activity (in vitro model), analgesic activity by tail-flick method, antibacterial activity by the agar diffusion and by MIC methods and antifungal activity.

Keywords: Chloro; Fluoro; Pyrazole; Benzothiazoles; Anti-bacterial; Analgesic; Anti-inflammatory activity

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INTRODUCTION

Pyrazole derivatives are known for their anti-inflammatory (in vitro), analgesic, ulcerogenic activities and acute toxicity [1,2]. There are plenty of reports regarding the pharmacological activities in the literature [3-10]. Encouraged by these reports in the literature regarding the pharmacological properties of pyrazole derivatives associated with benzothiazole heterocycles [11-16] and in continuation of our search for pharmologically potent compounds we undertook the synthesis of Chloro and fluoro substituted benzothiozoles linked with pyrazole moiety in the present investigation.

Pyrazoline and pyrazoles derivatives constitute an interesting class of organic compounds, which have associated with diverse chemical and pharmacological properties [17,18]. These compounds are known for their antitumor, analgesic, anti-inflammatory, insecticidal, antiarthritic, cerebroprotective effect and antidepressant properties [19-22]. Several substituted pyrazolines and pyrazoles are found to be effective bleaching agents, luminescents and fluorescents [23]. They are also useful as biodegradable agrochemicals [24].

The biological properties of fluorine and chlorine containing compounds have been recently investigated. Owing to their unique properties, such as high thermal stability and lipophilicity, fluoro-organic compounds have been frequently used as biorelated materials, medicines and agrochemicals [25,26].

It could be noted from the literature the heterofused benzothiazoles in general and the fused pyrazoline and pyrazole benzothiazoles, in specific are associated with varied biological properties. It is also evident that relatively a very few compounds of chloro fluoro benzothiazoles associated with pyrazoline and pyrazole hetrocycles are so far in spite of their significance.

THEORY

By the support of the above mentioned factors and in continuation of our work on fluro-chloro benzothiazoles, it has been considered worthwhile to effect the synthesis of some new 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl]-6-fluro-1,3-benzothiazoles (5a-h) and 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazoles (6a-h). For this purpose, the intermediate 7-chloro-6-fluoro-benzothiazol-2-ylhydrazine (3) has been synthesized by unambiguous method and made use of, as specified in Scheme I.

The required 2-hydrazinobenzothiazole (3) was prepared by the action of 2-amino-7-chloro-6-fluro benzothiazole and hydrazine hydrate in ethylene glycol under the influence of concentrated hydrochloric acid MP 228°C.

Similarly, different chalcones (4a-h) have been obtained by the condensation of benzaldehyde or different substituted benzaldehyde with p-chloro acetophenone in the presence of alcoholic sodium hydroxide and identified by their literature melting points [18].

Reaction of 7-Chloro-6-Fluoro Benzothiazol-2-yl Hydrazine with Chalcones (4a-h)

The compound 7-chloro-6-fluorobenzothiazol-2-yl hydrazine (3) has been subjected to cyclodehydration with different chalcones (4a-h). The chalcones for this purpose were prepared by refluxing compound 3 and 4a-h in ethanol under the influence of glacial acetic acid gave 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazoles (5a-h) in good yields. This has been purified by recrystallization from ethanol and subjected for analytical and spectral analysis. The compounds obtained from all above reaction have

been characterized as corresponding 7-chloro-2-[3-(4-chlorophenyl)-5aryl-4,5-dihydro-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazoles (5a-h).

Infrared-spectrum of the compound 5b (in Nujol) has been found to exhibit characteristic absorption frequencies at 2940 (-CH), 1620 (C=N), 1320 cm^{-1} (C-F). Further, the spectrum has shown the absence of bands characteristic of -$NHNH_2$ group. ^{1}HNMR Spectrum of the compound 5b (in $CDCl_3$) has showed characteristic proton signals at 1.164-1.252 (t, 3H, OCH_3), 3.604-3.621 (dd, 1H, C_4-Ha), 3.944-3.960 (dd, 1H, C_4-Hb), 6.876-6.904 (m, 1H, C_5–H), 7.042-8.0411 (m, 9H, Ar-H), 8.958 δ (s, 1H, -OH) (Table 1).

Mass spectrum of the compound 5b, has recorded its molecular ion peak at 489 (100%) M^+, exactly equivalent mass of the assigned structure. Based on the spectral data, the compound has been characterized as 5-[1-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl]-2-methoxyphenol (5b).

Similarly, the 7-chloro-6-fluorobenzothiazol-2-yl hydrazine (3) has been subjected to cyclodehydration with the rest of eight chalcones (4a-h). The products obtained in each such reaction has been characterized as corresponding 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-4, 5-dihydro-1-pyrazol-1-yl]-6-fluro-1,3-benzothiazole (5a-h). Physical, analytical and spectral data of the synthesized compounds are presented in the Tables 2a and 2b.

Reaction of 7-Chloro-2-[5-Aryl-3-(4-Chlorophenyl)-4,5-Dihydro-1H-Pyrazol-1-Yl]-6-Fluoro-1,3-Benzothiazole with Iodobenzenediacetate (IBD)

Catalytic dehydrogenation of pyrazolines using iodobenzenediacetate as a oxidizing agent convert the pyrazolines in to corresponding pyrazoles. The pyrazolines (5a-h) were stirred with one equivalent of iodobenzenediacetate in dichloromethane at the room temperature for 4-5 h. The reaction smoothly gave the desired 7-chloro-2-[5-aryl-3-(4-chlorophenyl)-1H-pyrazol-1-yl]-6-fluro-1,3-benzothia-zoles (6a-h).

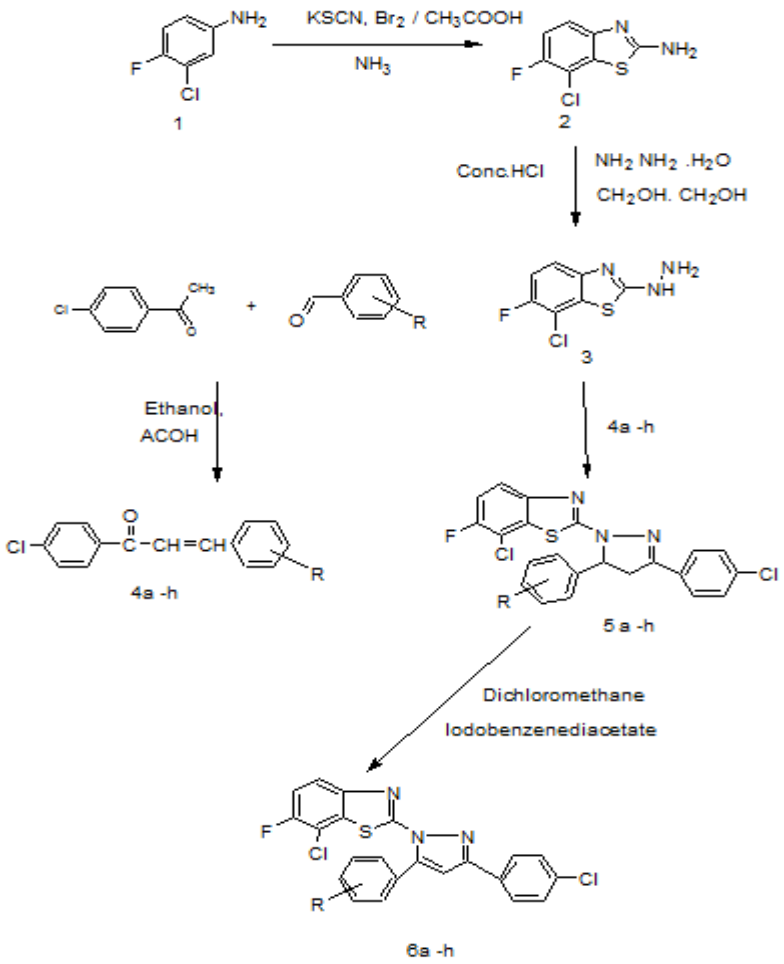
For example 5-[1-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl]-2-methoxyphenol (5b) was treated with equivalent amount of iodobenzene diacetate (IBD) in dichloromethane at the room temperature for 5 h has resulted in the formation of product. It has been purified by recrystalization from ethanol to obtain a crystalline solid, m.p. 125°C.

Infrared-spectrum of the compound (5b) (in Nujol) has been exhibited characteristic absorption frequency (cm-1) at 2950 (-CH), 1600 (C=N) and 1310 cm-1 (C-F). 1HNMR Spectrum of the compound (5b) (in CDCl3) it has showed characteristic proton signal (in δ, ppm) at1.253 (s, 3H, OCH3), 6.998 (s,1H, C4-H), 7.091-7.15 (m, 9H, Ar-H) and 9.750 (s, 1H, -OH), Mass spectrum of the compound has recorded its molecular ion peak at m/z 486 (100%) as its base peak and this happens to be in agreement with the mass number of the assigned of the structure.

Based on the spectral data the compound obtained could, thus be characterized as 5-[1-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-3-(4-chlorophenyl)-1H-pyrazol-5-yl]-2-methoxyphenol (6b)

Similarly the 7-chloro-2-[5aryl-3-(4-chlorophenyl-4,5-dihydro-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazoles (5a-h) have been subjected to oxidation or catalytic dehydrogenation with equivalent amount of iodobenzenediacetate. The products obtained in each case has been characterized as the corresponding 7-chloro-2-[5-aryl-3-(4-chlorophenyl)-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazoles (6a-h).

Their physical, analytical and spectral data of all these compounds are presented in the Tables 3a and 3b.



Scheme 1. 5a-h & 6a-h, R=H, 4-Cl, 4-CH_3, 4-OCH_3, 3-OCH_3, 2-Cl, (4-OCH_3, 3-OH), 3-Cl

EXPERIMENTAL PROCEDURES

Melting points were determined in open capillaries and are uncorrected. The purity of all the synthesized compounds was checked by TLC, IR spectra were recorded on Shimadzu FTIR 8400S by using KBr disc method and the NMR spectra were recorded on AV-400 Instrument

Synthesis of 7-Chloro-6-Fluoro-2-Amino-1,3-Benzothiazole (2)

To glacial acetic acid (20 ml) cooled below room temperature were added 8 g (0.08 mol) of potassium thiocyanate and 1.45 g (0.01 mol) of fluoro chloro aniline. The mixture was placed in freezing mixture of ice and salt and mechanically stirred while 1.6 ml of bromine in 6.0 ml of glacial acetic acid was added, from a dropping funnel at such a rate that the temperature never rise beyond room temperature. After all the bromine was added (105 min), the solution was stirred for 2 hours below room temperature and at room temperature for 10 h. The reaction mixture was then allowed to stand overnight, during which period an orange precipitate settled at the bottom. To this water (6 ml) was added quickly and the slurry was heated at 85°C on a steam bath and filtered hot. The resulting orange residue was placed in a reaction flask and treated with 10 ml of glacial acetic acid, heated again to 85°C and filtered hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution to pH 6. A dark yellow

precipitate was collectedand recrystallized from benzene, after treatment with animal charcoal gave yellow plates of 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole (2). After drying in an oven at 80°C, the dry compound (1 g 51.02%) melted at 210-212°C.

Synthesis of 7-Chloro-6-Fluoro-2-Hydrazino-1,3-Benzothiazole (3)

Concentrated hydrochloric acid (10 ml) was added drop wise with stirring to hydrazine hydrate (0.2 mol) at 5-10°C followed by ethylene glycol (40 ml). To the above solution, 7-chloro-6-fluoro-2-aminobenzothiazole (0.01 mol) was added in portions and resultant mixture was refluxed for 7 h and cooled. The solid separated was crystallized from aqueous ethanol, (yield 76%), melted at 228-230°C.

General Procedure for the Synthesis of Chalcones (4)

A mixture of sodium hydroxide (20 ml, 10%) and 100 ml ethanol was placed in 500 ml bolt-head flask provided with mechanical stirrer. The flask was immersed in a bath of crushed ice-bath. To the above mixture freshly distilled p-chloro acetophenone (5.2 g, 0.04 mole) was poured then started stirring with the addition of benzaldehyde (4.6 g. 0.043 mol). The temperature of the reaction mixture was maintained at 25°C with continuous stirring for 1 h. The contents of the reaction were kept overnight in deep-freezer. Then the product was filtered on suction pump, washed with cold water until washing were neutral to litmus. The crude chalcone (4a) was recovered after drying. Thus compound 4a was obtained in good yields, which was recrystallized from aq. ethanol (8.8 g, 80%, melts at 54°C). Similarly, different chalcones (4b-h) have been obtained by the condensation of different substituted benzaldehydes with p-chloro acetophenone in the presence of alcoholic sodium hydroxide.

General Procedure for Synthesis of 7-Chloro-2-[3-(4-Chlorophenyl)-5-Phenyl-4,5-Dihydro-1H-Pyrazol-1-Yl]-6-Fluro-1,3-Benzothiazoles (5a-H)

A mixture of 7-chloro-6-fluoro-2-hydrazino-1,3-benzothiazole (3) (2.02 g, 0.01 mol) and chalcones (4a-h) (0.01 mol) was refluxed for 2 h in ethanol (20 ml) containing few drops of acetic acid, kept at room temperature for 4-5 h. Separated solid was filtered, washed with water, dried and crystallized from ethanol (5a-h). The physical data in Table 1 and analytical data of all these compounds are tabulated in Table 2.

General procedure for Synthesis of Synthesis of 7-Chloro-2-[3-(4-Chlorophenyl)-5-Phenyl-1H-Pyrazol-1-yl]-6-Fluoro-1,3-Benzothiazoles (6a-h)

A solution of pyrazoline 5a-h (0.001 mole) in dichloromethane (20 ml) was added to iodobenzene diacerate (IBD) (0.0012 mol) was stirred at room temperature for 4 h. Dichloromethane was distilled off on a steam bath to give a gummy product which was triturated with petroleum ether to remove IBD and then was purified by recrystallization from ethanol to afford the title products (6a-h). The physical data in Table 1 and analytical data of all these compounds are tabulated in Table 2.

Table 1. Physical and analytical data of 7-chloro-2-[3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1 h-pyrazol-1 yl]-6-fluro-1,3-benzothiazole (5a-h) and Physical and analytical data of new 7-chloro-2[3-(4-chlorophenyl)-5-phenyl-1H-pyrazole-1-yl]-6-fluoro-1, 3-benzothiazole (6 a-h)

Sl. No.	Comp code	R	MP	Yield %	Molecular Formula	Mol. weight	C%	H%	N%
1	5a	H	98°C	65.55	$C_{22}H_{14}Cl_2FN_3S$	442	59.74	3.19	9.50
2	5b	4-OCH_3, 3-OH	240°C	59.5	$C_{23}H_{16}Cl_2FN_3O_2S$	488	56.57	3.3	8.60
3	5c	3-OCH_3	92°C	69.4	$C_{23}H_{16}Cl_2FN_3O_2S$	472	58.48	3.41	8.90
4	5d	4-OCH_3	84°C	60.20	$C_{23}H_{16}Cl_2FN_3OS$	472	58.48	3.41	8.90
5	5e	4-Cl	112°C	65.89	$C_{22}H_{13}Cl_3FN_3S$	477	55.42	2.75	8.81
6	5f	2-Cl	126°C	58.28	$C_{22}H_{13}C_3lFN_3S$	477	55.42	2.75	3.98
7	5g	3-Cl	80°C	66.82	$C_{22}H_{13}C_3lFN_3S$	477	55.42	2.75	8.81
8	5h	4-CH_3	120°C	70.23	$C_{23}H_{16}Cl_2FN_3S$	456	60.53	3.53	9.21
9	6a	H	120°C	59.25	$C_{22}H_{12}Cl_2FN_3S$	440	60.01	2.75	9.54
10	6b	4-OCH_3, 3-OH	201°C	70.91	$C_{23}H_{14}Cl_2FN_3O_2S$	486	58.73	3.4	8.93
11	6c	3-OCH_3	90°C	62.45	$C_{23}H_{14}Cl_2FN_3O_2S$	470	58.73	3.0	8.93
12	6d	4-OCH_3	125°C	66.7	$C_{23}H_{14}Cl_2FN_3OS$	470	58.73	3.0	8.93

13	6e	4-Cl	105°C	58.92	$C_{22}H_{11}Cl_3FN_3S$	475	55.66	2.34	8.85
14	6f	2-Cl	101°C	60.28	$C_{22}H_{11}C_3lFN_3S$	475	55.66	2.34	8.85
15	6g	3-Cl	113°C	67.8	$C_{22}H_{11}C_3lFN_3S$	475	55.66	2.34	8.85
16	6h	4-CH_3	85°C	63.63	$C_{23}H_{11}Cl_2FN_3S$	454	60.80	3.1	9.25

Table 2. IR, ^{1}H NMR and Mass Spectral data of substituted new 7-chloro-2-[3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]-6-fluro-1,3-benzothiazole (5a-h) and 7-chloro-2-[3-(4-chlorophenyl)-5-phenyl-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazole (6 a-h)

Comp. code	Molecular weight	I.R. Spectral Data (in cm^{-1})				^{1}H NMR (in δ ppm) and Mass Spectral data
		R	C=N	C-F	C-Cl	
5a	442	-H	1610	1250	730	---------
5b	488	-OCH_3 -OH	1260	1320	735	1.164 -1.252(t, 3H, OCH_3), 3.604 - 3.621(dd, 1H, C_4- Ha), 3.944 - 3.960(dd, 1H, C_4-Hb), 6.876 - 6.904(m, 1H, C_5–H), 7.042 - 8.0411 (m, 9H, Ar-H), 8.958 (S 1H, -OH) MS : m/z 489 (M^+ +1), 491(M^++3)
5c	472	-OCH_3	1600	1270	740	----------
5 d	472	–OCH_3	1610	1260	730	1.252(S, 3H, OCH_3), 3.749 - 3.770(dd, 1H, C4 –Ha), 3.901-3.904(dd, 1H, C_4-Hb), 6.879 - 6.956(m, 1H, C_5 -H), 7.263-7.969 (m, 10H, Ar-H)
5 e	477	–Cl	1620	1260	740	3.604-3.621(dd, 1H, C_4-Ha), 3.944 – 3.960(dd,1H,C_4-Hb), 6.724 (d, 1H, C_5-H), 6.992 -7.909(m, 10H, Ar-H)
5 f	477	–Cl	1630	1280	745	3.173 -3.192(dd, 1H, C_4-Ha), 3.697-3.705(dd, 1H, C_4-Hb), 6.049 - 6.102(m, 1H, C_5-1H) MS: m/z 478 (M^++1)
5 g	477	–Cl	1625	1320	725	----------

5 h	456	$-CH_3$	1610	1310	720	1.252(S, 3H, CH_3), 3.329 - 3.346(dd, 1H, C_4-Ha), 3.978 - 3.993(dd, 1H, C_4-Hb), 6.771(S,1H, C_5-H), 7.042 -7.978(m, 10H, Ar-H)
6 a	440	–H	1610	1255	730	---
6 b	486	$-OCH_3$ –OH	1600	1310	740	1.253(S, 3H, OCH_3), 6.998(S,1H, C_4 -H), 7.091 - 7.15 (m, 9H, Ar-H), 9.750(S, 1H, -OH), MS: m/z 486(M^+)
6 c	470	$-OCH_3$	1620	1260	730	---
6 d	470	$-OCH_3$	1650	1260	740	1.252 (S, 3H, OCH_3), 6.748 (S,1H,C_4-H), 6.992-8.047 (m,10H,Ar-H)
6 e	474	–Cl	1630	1250	750	6.789 (S, 1H, C_4-H), 6.796-7.982 (m, 10H, Ar-H)
6 f	474	–Cl	1615	1295	720	6.451 (S, 1H,C_4-H), 6.809- 7.979 (m, 10H, Ar-H), MS: m/z 473 (M^+)
6 g	474	–Cl	1620	1310	735	---
6 h	454	$-CH_3$	1620	1320	740	1.936(S, 3H, CH_3), 6.774 (S, 1H, C_5-H), 7.172 - 7.823,(m, 10, Ar-H)

ANTI-INFLAMMATORY ACTIVITY (IN VITRO MODEL)

Many in vitro assays, each based on a specific biochemical or cellular mechanism have been developed for the initial screening of the anti-inflammatory compounds. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins as an in vitro screening model for anti-inflammatory compounds.

The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique, which was studied according to Muzushima and Kabayashi with slight modification. The standard drug and test compounds were dissolved in minimum amount of dimethylformamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different concentrations of drugs was mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at 27 ± 1°C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1°C on water bath for 10 min. After cooling the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The ibuprofen and diclofenac sodium was used as standard drug.

% of inhibition=100 X Vt /Vc, Where, vt=mean absorbance value of test group,

vc=mean absorbance value of control group.

Results for anti-inflammatory activity (*in vitro*) are given in the Table 3.

Table 3. Anti-inflammatory activity (in vitro model) of compounds (5 a –h) and **(6 a-h)**

Sl. No.	Compound code	Absorbance value (Mean ± SE)	Inhibition of denaturation (in%)
1	Control	0.046 ± 0.0005	--
2	Standard (Diclofenac sodium)	0.081 ± 0.0014	77%
3	5 a	0.058 ± 0.0003	27%
4	5 b	0.074 ± 0.0003	62%
5	5 c	0.054 ± 0.0008	19%
6	5 d	0.064 ± 0.0003	40.43%
7	5 e	0.051 ± 0.0005	10.86%
8	5 f	0.058 ± 0.0003	26.08%
9	5 g	0.069 ± 0.0005	50%
10	5 h	0.067 ± 0.005	45.65%
11	6 a	0.07 ± 0.0003	52.17%
12	6 b	0.066 ± 0.0015	43.47%
13	6 c	0.060 ± 0.0005	30.46%
14	6 d	0.071 ± 0.0003	54.34%
15	6 e	0.062 ± 0.0008	36.95%
16	6 f	0.063 ± 0.0003	39.13%
17	6 g	0.065 ± 0.0012	42.60%
18	6 h	0.054 ± 0.0012	17%

ANTI-INFLAMMATORY ACTIVITY (IN VIVO MODEL)

Anti-inflammatory Activity by Carrageenan Induced Rat Hind Paw Oedema Method

Animals were divided into control, standard, different test groups comprising of five animals in each group. They were fasted overnight with free access to water before experiment. In all groups, acute inflammation was produced by sub-plantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in the right hind paw of the rats and paw of the rats and paw volume was measured plethysmometrically at 0 h and 3 h after carrageenan injection. The test compounds (50 mg/kg) was administered orally, standard group was treated with diclofenac (50 mg/kg) orally 1 h before by injection and control group received only vehicle. Mean difference in paw volume was measured statically by student't' test in dunnett and percentage inhibition was calculated by following formula:

$$\%\ inhibition\ of\ \ edema = \frac{Vc - Vt}{Vc} \times 100$$

where, Vt=mean paw volume of test group, Vc=mean paw volume of control group.

Results for anti-inflammatory activity (in vivo model) are given in the Table 4.

Table 4. Anti-inflammatory activity (in-vivo model)

Sl. No.	Compound code	Dose: mg/kg	Mean difference in Paw volume ± SE after 3 h (ml)	% of inhibition
1	Control	--	3.59 ± 0.057	--
2	Standard (Diclofenac sodium)	50	1.05 ± 0.029***	70.75%
3	5b	50	2.22 ± 0.075**	41.78%
4	5d	50	2.09 ± 0.071**	38.16%
5	5g	50	2.28 ± 0.080**	36.49%
6	5h	50	2.12 ± 0.096**	40.94%
7	6a	50	2.16 ± 0.082**	38.83%
9	6b	50	2.09 ± 0.092**	41.78%
10	6d	50	2.12 ± 0.073**	40.94%
11	6g	50	2.17 ± 0.103**	39.55%

****P<0.01, ***P<0.001 when compared to control group**

ANALGESIC ACTIVITY (BY TAIL-FLICK METHOD)

Numbers of Albino rats were weighed. Basal reaction time to radiant heat is taken by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail-withdrawal from the heat (flicking response) was taken as the end point. Normally a mouse withdraws its tail within 3-5 s. A cut off period of 10-12 s is observed to prevent damage to the tail. Any animal failing to withdraw its tail within 3-5 s was rejected from study. At least 3-5 basal reaction times for each mouse at the gape of 5 min were taken to confirm normal behavior of the animal. Aspirin is injected and noted the reaction time at 5, 15, 30, and 60 min after the drug administration. As the reaction time reaches 10 s it is considered maximum analgesia and the tail is removed from the source of heat to avoid the tissue damage.

Percentage increase in reaction time (index of analgesia) at the each time interval was calculated. Mean difference was measured statically by student 't' test dunnett and Percentage protection was calculated using the formula:

$$\% \ protection = \frac{Vt - Vc}{Vc} \times 100$$

Where, Vc=Mean tail time flick of control group. Vt=Mean tail flick time of test group.

Results for analgesic activity are given in the Table 5.

Table 5. Analgesic activity (By Tail-flick method)

Sl No	Compound code	Dose mg/kg	Tail-flick (Values in sce.) (Mean± SE)	Percentage of protection
1	Control	--	3.66 ± 0.2404	--
2	Diclofenac Sod.	50	6.8 ± 0.01***	85%
3	5*b*	50	5.83 ± 0.166**	59.28%
4	5*d*	50	6.2 ± 0.321***	74.86%
5	5*g*	50	4.73 ± 0.371*	29.23%
6	5*h*	50	5.033 ± 0.491*	37.43%
7	6*a*	50	5.5 ± 0.288**	50.27%
8	6*b*	50	6.36 ± 0.29**	73.77%
9	6*d*	50	5.33 ± 0.333*	45.62%

****P<0.01, ***P<0.001 when compared to control group.**

ANTIBACTERIAL ACTIVITY (THE AGAR DIFFUSION METHOD)

The antibacterial activity of the synthesized compounds was studied, systematically against four different strains of bacteria [Gram (+ve) and Gram (-ve)] by the agar diffusion method. The specific method adopted in the present investigation was cup-plate method involving cups of standard diameter, the nutrient agar medium and containing standard bacterial inoculum. The test compounds were introduced into the cups and the diameters of the zones of inhibition were measured.

All the test compounds were evaluated for antibacterial activity against *Staphylococcus aureus* [Gram (+ve)], *Escherichia coli*[Gram (-ve)]and *streptococci*[Gram (+ve)], *Pseudomonas* [Gram (-ve)], following the agar diffusion method of assays. The results for antibacterial activity are given in the Table 6.

Table 6. Antibacterial activity (By cup-plate method)

Sl. No.	Compound Code	Mean zone of inhibition in (mm)							
		***Streptococci* (G+ve)**		***Pseudomonas* (G-ve)**		***Staphylococcus aureus* (G+Ve)**		***Escherichia coli* (G-ve)**	
		50 µg	**100 µg**	**50 µg**	**100 µg**	**50 µg**	**100 µg**	**50 µg**	**100 µg**
1	Procaine Penicillin	21	23	--	--	21	23	--	--
2	Streptomycin	--	--	20	22	--	--	20	22
3	5a	13 (0.61)	15 (0.65)	16	18	11 (0.52)	12 (0.57)	12	13

				(0.80)	(0.81)			(0.60)	(0.59)
4	5b	15 (0.71)	18 (0.78)	14 (0.7)	16 (0.72)	16 (0.76)	20 (0.86)	16 (0.80)	18 (0.81)
5	5c	10 (0.47)	12 (0.52)	13 (0.65)	15 (0.68)	13 (0.61)	15 (0.65)	11 (0.55)	12 (0.54)
6	5d	14 (0.66)	16 (0.69)	14 (0.70)	16 (0.72)	14 (0.66)	18 (0.78)	13 (0.65)	15 (0.68)
7	5e	14 (0.66)	18 (0.78)	15 (0.75)	18 (0.81)	15 (0.7)	19 (0.82)	12 (0.60)	12 (0.54)
8	5f	11 (0.52)	14 (0.60)	10 (0.5)	10 (0.45)	12 (0.57)	15 (0.65)	12 (0.60)	15 (0.68)
9	5g	09 (0.52)	12 (0.52)	14 (0.70)	19 (0.86)	12 (0.57)	16 (0.69)	13 (0.65)	14 (0.63)
10	5h	14 (0.66)	16 (0.69)	12 (0.60)	15 (0.68)	14 (0.66)	18 (0.78)	12 (0.60)	13 (0.59)
11	6a	12 (0.57)	16 (0.69)	10 (0.5)	12 (0.54)	12 (0.57)	14 (0.60)	12 (0.60)	14 (0.63)
12	6b	14 (0.66)	15 (0.65)	16 (0.80)	17 (0.77)	13 (0.61)	17 (0.73)	14 (0.70)	16 (0.72)
13	6c	13 (0.61)	19 (0.82)	14 (0.70)	17 (0.77)	13 (0.61)	17 (0.73)	11 (0.55)	13 (0.59)
14	6d	14 (0.66)	17 (0.73)	13 (0.65)	14 (0.63)	14 (0.66)	17 (0.73)	13 (0.65)	16 (0.72)
15	6e	14 (0.66)	16 (0.69)	12 (0.60)	14 (0.63)	12 (0.57)	16 (0.72)	14 (0.70)	20 (0.90)
16	6f	12 (0.57)	16 (0.69)	11 (0.55)	14 (0.63)	14 (0.66)	18 (0.65)	11 (0.55)	13 (0.59)
17	6g	11 (0.52)	14 (0.60)	15 (0.75)	19 (0.86)	12 (0.57)	15 (0.65)	11 (0.55)	17 (0.77)
18	6h	13 (0.61)	15 (0.65)	15 (0.75)	17 (0.77)	11 (0.52)	13 (0.56)	14 (0.70)	17 (0.77)

Std: Procaine Penicillin and Streptomycin

Mean zone of inhibition is including bore diameter, Bore diameter is 8 mm.

$$Activity\ index = \frac{Test\ compound}{Standard\ compound}$$

ANTIBACTERIAL ACTIVITY (BY MIC METHOD)

The antibacterial activity of synthesized compounds were tested *in vitro* on strains of four microorganisms, *Escherichia coli, Streptococci, Pseudomonas typhii, Staphylococcus aureus.*

The antibacterial activity was evaluated by tube dilution method (turbidometric method). The turbidometric method depends upon the inhibition of growth of microbial culture in a uniform solution of antibacterial in a fluid medium that is favorable to its rapid growth in the absence of the antibacterial agent. In this method minimal inhibitory concentration (MIC) of the lowest concentration of an antibacterial agent that inhibits the growth of test organism can be detected. The synthesized compounds were dissolved in DMF to prepare a stock solution of 1 mg/ml conc. with this stock solution different dilutions 800 µg to 5 µg/ml were prepared. The ciprofloxacin was also prepared in DMF to obtain a conc. of 800 µg/ml to 5 µg/ml.

Preparation of Double Strength Nutrient Media Composition

Peptone:1 g, Yeast: 0.3 g, Sodium Chloride: 0.5 g, Distilled water: 50 ml.

The solid ingredients were dissolved in water and pH was adjusted to 7.4 ± 0.2 and the media was sterilized by autoclaving at 15 lb/psi for 15 mins.

Preparation of suspension of microorganism: Transferring the microorganism from culture to 5 ml of sterile normal saline (0.09%) solution made of each microorganism.

Determination of Minimal Inhibitory Concentration

The sterile test tube containing 1 ml of sterile media were added with 1 ml of different serially diluted test samples. To these tubes 0.1 ml of normal saline solution suspended with respective microorganisms were inoculated and incubated at 37 ± 2°C for 18 to 24 h. The growths in the tubes were observed visually for turbidity and inhibition was determined by lowest concentrations of sample that prevented the development of turbidity. The procedure was repeated to confirm the MIC. The antibacterial screening results for the determination of MIC are given in the Table 7.

Table 7. Antibacterial activity (by MIC method)

Sl. No.	Compound code	Staphylococcus aureus (G+ve)	Streptococci (G+ve)	Escherichia coli (G-ve)	Pseudomonas (G-ve)
1	5b	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
2	5d	50 µg/ml	100 µg/ml	100 µg/ml	50 µg/ml
3	5e	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml
4	5f	100 µg/ml	200 µg/ml	100 µg/ml	100 µg/ml
5	5h	200 µg/ml	400 µg/ml	200 µg/ml	50 µg/ml
6	6b	100µg/ml	100 µg/ml	200 µg/ml	50 µg/ml
7	6c	200 µg/ml	50 µg/ml	400 µg/ml	200 µg/ml
8	6e	400 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
9	6f	50 µg/ml	100 µg/ml	400 µg/ml	50 µg/ml
10	6h	100µg/ml	400 µg/ml	50 µg/ml	200 µg/ml

ANTIFUNGAL ACTIVITY

All those compounds screened for antibacterial activity were also tested for their antifungal activity agar diffusion method. The fungi employed for screening were: *Asperigillus flavus* and *Candida albicans*. The results for antifungal activity are given in the Table 8.

Table 8. Antifungal activity

Sl. No.	Compound code	Mean zone of inhibition in (mm)			
		Candida albicans		***Aspergillus flavus***	
		50 µg	**100 µg**	**50 µg**	**100 µg**
1	Griseoflavin	20	22	19	23
2	5a	14 (0.70)	17 (0.77)	12 (0.63)	15 (0.65)
3	5b	16 (0.8)	19 (0.83)	14 (0.73)	17 (0.73)
4	5c	16 (0.8)	19 (0.83)	14 (0.73)	19 (0.82)
5	5d	15 (0.75)	17 (0.77)	17 (0.89)	19 (0.82)
6	5e	12 (0.6)	14 (0.63)	11 (0.57)	13 (0.56)
7	5f	13 (0.65)	16 (0.72)	16 (0.84)	20 (0.86)
8	5g	12 (0.6)	14 (0.63)	11 (0.57)	13 (0.56)
9	5h	10 (0.50)	13 (0.59)	12 (0.63)	12 (0.52)
10	6a	15 (0.75)	15 (0.68)	12 (0.63)	14 (0.60)
11	6b	17 (0.85)	19 (0.86)	16 (0.84)	18 (0.78)
12	6c	14 (0.70)	16 (0.72)	12 (0.63)	13 (0.56)
13	6d	18 (0.9)	21 (0.95)	14 (0.73)	17 (0.73)
14	6e	16 (0.8)	19 (0.86)	10 (0.52)	12 (0.52)
15	6f	10 (0.5)	11 (0.5)	13 (0.68)	14 (0.69)
16	6g	15 (0.75)	17 (0.77)	14 (0.73)	17 (0.73)
17	6h	12 (0.6)	15 (0.68)	13 (0.68)	14 (0.60)

Standard: Griseofulvin (Grisovin FP)

Mean zone of inhibition is including bore diameter, Bore diameter is 8 mm.

$$Activity\ index = \frac{Test\ compound}{Standard\ compound}$$

RESULTS AND DISCUSSION

Anti-inflammatory Activity (*In Vitro* Model)

All the synthesized compounds of 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazole (5a-h) and 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazole (6a-h) have been evaluated for anti-inflammatory (*in-vitro*) activity by bovine albumin serum denaturation method. The results are presented in Tables 3 and 4. Among the tested compounds 5b, 5d, 5g,5h showed 62%, 40.43%, 50%, 45.68%, and 6a, 6b, 6d, and 6g showed 52.17%, 43.47%, 54.34%, 42.60%, inhibition of albumin denaturation compare to standard drug diclofenac sodium which showed inhibition of 77%. Since these compounds have shown more than 40% inhibition of bovine albumin serum as per the literature all these compounds where selected for *in vivo* evaluation of anti-inflammatory activity against albino rats.

Anti-inflammatory Activity (*In Vivo* Model)

The selected compounds of 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-4,5-dihydro-1*H*-pyrazol-1-yl]-6-fluoro-1,3-benzothiazoles (5) and 7-chloro-2-[3-(4-chlorophenyl)-5-aryl-1*H*-pyrazol-1-yl]-6-fluoro-1,3-benzothiazoles (6) have been evaluated for anti-inflammatory activity by carrageenan induced rat hind paw oedema method. The results are presented in Tables 3 and 4 Diclofenac sodium was used as standard drug.

The tested compounds 5b (41.78%), 5d (38.16%), 5g (36.47%), 5h (40.94%), 6a (39.83%), 6b (41.78%), 6d (40.94%) and 6g (39.55%) showed moderate anti-inflammatory activity, compare to standard diclofenac sodium (70.75%).

Analgesic Activity

Compounds which were selected for in vivo anti-inflammatory activity that is5b, 5d, 5g, 5h, 6a, 6b, 6d, and 6gwere also screened for analgesic activity by tail –flick method. The results are presented in Table 5. Diclofenac sodium was used as standard drug which exhibited 85% protection.

The tested compounds 5b (59.28%),5d (74.86%), 5g (29.23%), 5h (37.83%), 6a (50.27%), 6b (73.77%), 6d (45.62%) and 6g (54.64%), showed moderate (protection) analgesic activity compare to standard diclofenac sod.85%.

From this it was concluded that the tested compounds have shown moderate analgesic activity in comparison with standard drug, diclofenac sodium.

Antibacterial Activity (by Cup-Plate Method)

Synthesized compounds have been evaluated for antibacterial activity at concentration of 50 μg/ml and 100 μg/ml by standard method, against following bacteria:

a) (i) *Staphylococcus aureus* (ii) *Streptococci* Gram (+ve).

b) (i) *Pseudomonas*, (ii) *Escherichia coli* Gram (-ve).

All the compounds have been shown to exhibit a moderate broad spectrum of activity. The test compounds have been found to active against both gram (+ve) and gram (-ve) organisms.

The test compounds 5b, 5d, 5e, 5f, 5h, 6b, 6c, 6e, 6f, and 6h showed superior in it's antibacterial activity at lower (50 μg/ml) and higher (100 μg/ml) concentration against *Streptococci* Gram (+ve), compare to standard drug procaine penicillin.

Compounds 5a, 5b, 5d, 5e, 5g, 6b, 6c, 6d, 6g and 6h showed Promising antibacterial activity against *Pseudomonas* Gram (-ve), bacteria compare to standard drug streptomycin.

Compounds 5b, 5d, 5e, 5h, 6b, 6c, 6d, and 6f, showed moderate activity against *Staphylococcus aureus* gram (+ve) bacteria compare to standard drug procaine penicillin.

Perusal of Table 6 reveals that compounds 5b, 5d, 5f, 5g, 6b, 6d, 6e and 6h being same has been found exhibit relatively by more in their inhibitory action against *Escherichia coli* Gram (-ve) bacteria, compare to standard drug streptomycin.

Anti-bacterial Activity (by MIC Method)

Among the compounds, the compounds which have shown highest actvivity were selected for evaluation of antibacterial activity by MIC method. The compounds 5*b*, 5d, 5e, 5f, 5h, 6b, 6c, 6e, 6f and 6h have shown varied antibacterial response to the organism tested. The results are presented in Table 7.

The *Staphylococcus aureus* Gram (+ve) organism responded very sensitively to compounds 5d and 6f have shown the MIC activity at 50 μg/ml, the organism found to be sensitive to compound 5e,5f,6b and 6h have shown the MIC activity at 100 μg/ml. Remain compounds 5b, 5h, 6c and 6e, shown moderate or less MIC activity. Another Gram (+ve) organism *streptococci* found to sensitive to compound 5b,6c and 6ehave shown the MIC activity at 50 μg/ml. Remaining compounds 5d, 6b, and 6f shown moderate activity and shown MIC activity at 100 μg/ml respectively.

Escherichia coli Gram (-ve) organism found to be extremely sensitive to compounds 5e, 6h and 5b, 5d, 5f, 6e; it has shown the activity at 50 μg/ml and 100 μg/ml respectively. Another Gram (-ve) organism Pseudomonas found to very sensitive to compounds 5d, 5h and it has shown the activity at 50 μg/ml. Compounds 5eand 5ffound to sensitive and shown MIC activity at 100 μg/ml.

Staphylococcus aureus showed the MIC activity at 400 μg/ml for 6*e* compound. *Streptococci* found less sensitive to compound 5h and shown the activity at 400 μg/ml. *Escherichia coli* found less sensitive to compounds 5h, and 6c, 6f it shown activity at 200 μg/ml and 400 μg/ml. *Pseudomonas* also found less sensitive to compounds 5b, 6c, 6e and 6h it was shown activity at 200 μg/ml.

The variation observed in the antibacterial activity to the compounds tested may be due to variation in the strains used for the antibacterial studies.

Antifungal Activity

All the compounds were subjected to antifungal activity. For these activities *Candida albicans and* Aspergillus *flavus* fungal organisms were used. The Griseofulvin was used as standard. Standard and synthesized compounds were tested at two conc. viz., 50 μg/ml and 100 μg/ml. The experimental results are presented in Table 8.

The compounds have shown activity, but none of them have shown better activity than standard. Some of compounds like 5a, 5b, 5c, 5d, 5f, 6b, 6d, 6e and 6g have shown activity almost equal to standard against *Candida albicans* at 100 μg/ml conc. and 5b, 5c, 5d, 6b, 6d and 6e have shown activity near to standard at 50 μg/ml conc. and the compounds 5b, 5c, 5d, 5f, 6b, 6d and 6g have shown activity near to standard against *Aspergillius flavus*

organism at 100 µg/ml conc. and 5d, 5f and 6b at 50 µg/ml conc. The remaining compounds have shown very low activity. From this it is concluded that the synthesized compounds have shown better activity against *Aspergillus flavus* than *Candida albicans*.

CONCLUSION

Eight new compounds of 7-chloro-2-[3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazole (5a-h) and eight new compounds of 7-chloro-2-[3-(4-chlorophenyl)-5-phenyl-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazole (6a-h)were synthesized. All the synthesized compounds were characterized by IR, ^{1}HNMR and mass spectral properties.

The synthesized compounds were screened for anti-inflammatory and analgesic, antimicrobial, antifungal, activities. Tested compounds exhibited moderate to good antimicrobial activity against both Gram (+ve) and Gram (-ve) bacteria. A few of compounds exhibited significant antifungal activity compare to standard drug against *Candida albicans* and moderate against *Aspergillus flavus.*

Most of these compounds showed significant anti-inflammatory activity on both *in vitro* and *in vivo* models, comparing to standard. Some of these compounds exhibited good to significant analgesic activity comparing to standard. The compounds, which have been found to be more active, a detail further investigation required for exploitation.

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